

Quantification of Carbonyl Compounds in Oxidized Low-Linolenate, High-Stearate and Common Soybean Oils

Lynne A. Miller and Pamela J. White*

Food and Nutrition Department, 111 MacKay Hall, Iowa State University, Ames, IA 50011

The carbonyl compounds in five oxidized soybean oils (SBO) of various fatty acid compositions were determined. Three were from common normal soybean varieties, and two were from lines developed from new mutant varieties. One mutant line had a linolenate (18:3) content of 3.5% (A5), and one had a stearate (18:0) content of 24% (A6). SBO were stored at 28 C and 60 C. Trichlorophenylhydrazones (TCPH) of carbonyls formed during oxidation were quantified and tentatively identified by gas chromatography. The storage temperature and the composition of the oils affected the types and amounts of volatiles produced. Hexanal was the major volatile in the oils in both storage tests. After 60 C storage, 2- and/or 3-hexenal was present only in the oil with the highest 18:3 content (BSR 101, 18:3 = 9%). The amounts of the carbonyls formed in A5 were 2 to 5 times less than the amounts formed in BSR 101. The amounts of many of the carbonyls were converted into relative flavor potency by using reported data. Hexanal was the major contributor to flavor. After storage at 28 C, 2- and/or 3-hexenal was the second most intense flavor compound regardless of the 18:3 content of the oil. The amount of a compound and the threshold value did not always predict its flavor importance according to the flavor potency data.

The development of objectionable flavors and odors by oxidation has obvious detrimental effects on the quality and consumer acceptability of soybean oil (SBO) (1). The oxidative deterioration of SBO primarily involves autoxidative reactions, which are accompanied by various secondary reactions (2). These oxidative reactions result in the formation of a variety of decomposition products, some of which are volatile.

Although these volatile products represent only a minor portion of the total decomposition materials, they undoubtedly have an important impact on flavor (3). Frankel (1) summarized the volatile oxidation products that have been identified in homogeneous solutions of methyl hydroperoxides, fatty acids (FA) or triglycerides containing oleate (18:1), linoleate (18:2) or linolenate (18:3).

The nature and relative proportion of volatile oxidation products can be affected by temperature and secondary reactions. Interactions between different methyl esters of FA during oxidation of their mixtures have been demonstrated (4). When a mixture of equal amounts of 18:2 and 18:3 fatty esters was oxidized to a level of 10%, a greater ratio of 18:3/18:2 hydroperoxides was detected. At oxidation levels above 10%, a greater proportion of 18:2/18:3 hydroperoxides was detected. Hydroperoxides are the initial products of lipid oxidation. These rapidly break down into volatile and non-volatile products.

The volatile unsaturated aldehydes and ketones formed

as primary decomposition products from hydroperoxides are obvious sources of secondary volatile products because of their susceptibility to further oxidation. Both 2-nonenal and 2,4-heptadienal were more rapidly oxidized than methyl esters of either 18:2 or 18:3 (1).

The type of flavor perceived from a mixture of volatiles can depend on their complex interactions, concentration ranges and the medium in which they are tested. It is often assumed that the importance of a compound's contribution to flavor is proportional to its threshold level. To confirm this, Dixon and Hammond (5) compared the flavor intensities of many of the aldehydes and ketones commonly found in oxidized fats and oils and matched them to a standard scale of increasing flavor intensity. The compounds differed, not only in their threshold values, but also in the slopes of the concentration vs. flavor intensity. For example, to get the same increase in flavor intensity, the hexanal concentration had to be increased three times whereas the concentration of 2,4-nonadienal had to be increased nine times. The data also showed that threshold values did not predict a compound's relative importance at higher concentrations. When aldehydes were mixed together in the proportions found in oxidized SBO, the flavor of the synthetic mixture was similar to, but easily distinguishable from, oxidized SBO. Perhaps further complications arise from additive and antagonistic interactions between mixtures of volatiles (1). Therefore, the true contribution of each carbonyl to the flavor of a mixture is still questionable.

Ullrich and Grosch (6) measured the most intense and most abundant odor compounds formed during autoxidation of linoleic acid and methyl linoleate. Hexanal was the most abundant product formed from both compounds. The relative abundance of the other compounds found depended upon the form of 18:2 oxidized. However, 2-*trans*-heptenal, 2-*cis*-octenal and 2-*trans*-octenal were included in the five most abundant compounds from each form. The autoxidation time influenced which compound had the most intense odor. When linoleic acid was oxidized for 24 hr, 2-*cis*-nonenal was the most potent odor compound. After 48 hr, hexanal and 2-*cis*-nonenal contributed equally as the most intense odor compounds. After 72 hr, 2-*trans*-octenal became the most potent odor compound. The 2,4-decadienals were not important contributors to the intensity of the odor.

In a previous study (7), the oxidative stabilities of SBO from commercial varieties were compared with those of a low-18:3 SBO (A5) and a high-18:0 SBO (A6). The present study was undertaken to compare the amounts and types of carbonyls formed during oxidation of A5, A6 and commercial SBO.

EXPERIMENTAL PROCEDURES

Extraction, refining and deodorization. Soybean seeds from three varieties and two lines were grown at ISU. The oil was removed from the seed by cold-pressing

*To whom correspondence should be addressed.

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with a Hander Screw press (model H54, Osaka, Japan). For the 28 C storage study, 16 kg of seed from the 1984 crop of A5, A6, Pella and BSR 101 were pressed. For the 60 C storage study, 23 kg of seed from the 1985 crop of A5, A6, Hardin and BSR 101 were pressed. The commercial varieties, Pella, Hardin and BSR 101, were selected to represent the normal range of 18:3 found in soybean oil.

The oils were refined and deodorized under laboratory conditions as described previously (7). Immediately after deodorization, all oils were stored under nitrogen and held at -10 C until storage tests began. No additives were included in any oils.

Storage tests. Two storage tests were conducted. In test 1, oils from the 1984 crop were stored at 28 C. One hundred g of each oil (A5, A6, Pella and BSR 101) were stored in 150-ml beakers that were loosely covered. Aliquots were removed periodically and stored under nitrogen at -10 C until analyzed.

In test 2, duplicate lots of each oil from the 1985 crop (A5, A6, Hardin and BSR 101) were deodorized separately and stored at 60 C. Ninety g of each oil were stored in 100-ml beakers that were loosely covered. Aliquots were removed periodically and stored under nitrogen at -10 C until analyzed.

Chemical analyses. The trichlorophenylhydrazone (TCPH) derivatives of the carbonyls formed in each of the SBOs on the last day of storage were prepared according to a method by White and Hammond (8). A blank was prepared in the same manner as a sample, but without the addition of fat. A 0.5- μ l sample was injected into a Varian 3700 gas chromatograph (GC) (Sunnyvale, California) equipped with a flame ionization detector. A 30 m \times 0.32 mm i.d. fused silica capillary column coated with a nonpolar bonded phase (SPB-1, Supelco Inc., Bellefonte, Pennsylvania) was used.

The GC was temperature programmed from 40 C to 280 C at 10 C/min, followed by a final hold of 10 min. For quantification, sample peak heights were compared with an internal standard, 2-nonanone. The amounts were calculated as ppm in SBO. Compounds were tentatively identified by comparison with the retention times of known compounds. A series of 2-ketone-TCPHs (C4, C5, C7-C10) served as working standards. Selected aldehydes (formaldehyde, acrolein, propanal, butanal, pentanal, hexanal, 2-trans-hexenal and octanal) were tested, and their retention times recorded in relation to the 2-ketones.

RESULTS AND DISCUSSION

Detailed information on storage stabilities of the oils used in the current study as measured by methyl esters of the fatty acids, peroxide value (PV), conjugated dienoic acid value (CD) and sensory tests have been reported previously (7). A summary of the data from the chemical tests appears in Table 1 (28 C storage) and Table 2 (60 C storage). Sensory data on intensity of off-flavor were inconclusive, although A5 and A6 tended to develop less off-flavor than the other oils after storage at 28 C. Even fewer sensory differences between oils were noted in the 60 C test.

Test 1, 28 C storage. The chromatograms of the TCPH formed from the oils on day 67 of storage are

TABLE 1

Fatty Acid Composition of Fresh Soybean Oils and Conjugated Dienoic Acid Values (CD) and Peroxide Values (PV) After Storage for 67 Days at 28 C

Soybean oil type	Fatty acid %			CD %	PV (meq/kg)
	18:0	18:2	18:3		
A5	4.1	44.2	3.7	0.49	28.7
A6	24.0	40.0	6.3	0.57	28.3
Pella	4.2	54.1	7.2	0.80	64.3
BSR-101	4.4	53.8	9.1	1.04	100.6

TABLE 2

Fatty Acid Composition of Fresh Soybean Oils and Conjugated Dienoic Acid Values (CD) and Peroxide Values (PV) After Storage for 8 Days at 60 C

Soybean oil type	Fatty acid %			CD %	PV (meq/kg)
	18:0	18:2	18:3		
A5	4.6	48.0	4.2	0.69	38.7
A6	17.2	42.2	7.2	0.66	40.2
Hardin	4.6	51.4	7.3	0.74	45.8
BSR-101	4.6	55.1	9.2	0.90	50.0

presented in Figure 1. The amounts of interfering compounds found in the blank have been subtracted from the heights of the peaks shown in Figure 1. Table 3 shows the identities and quantities of the TCPH after subtraction of the blank. Twenty-eight compounds were found in Pella and BSR 101, but many of these were missing in A5 and A6. Hexanal was the most abundant compound found in A6, Pella and BSR 101, and acrolein was the most abundant compound in A5. The order of abundance of the rest of the carbonyls differed among the oils.

White and Hammond (8), as well as other researchers (9-11), also have found many of these compounds in oxidized SBO. The amounts of the carbonyls formed in A5 were generally 20-30% of the amounts formed in BSR-101.

The amounts of many of the carbonyls found in the oxidized oils were converted into relative flavor potency by using data of Dixon and Hammond (5). By this conversion, the four most important flavor contributors for each of the oils in the current study are listed in Table 4.

Because several of the carbonyls found in the current study were not identified and Dixon and Hammond (5) did not measure the flavor intensities of carbonyls below C5 or above C10, the relative flavor potency could not be calculated for all the carbonyls found. Thus, the flavor intensity rank of the carbonyls shown in Table 4 is only tentative. The minor flavor components also could affect the flavor character of the mixture. Dixon and Hammond (5) tested the flavor intensity of single compounds but, as they and other researchers (1) have discussed, the additive and antagonistic effects of mixtures or matrix effects have not been fully explored.

Test 2, 60 C storage. The chromatograms of the TCPH formed from the oils on day 8 of storage after

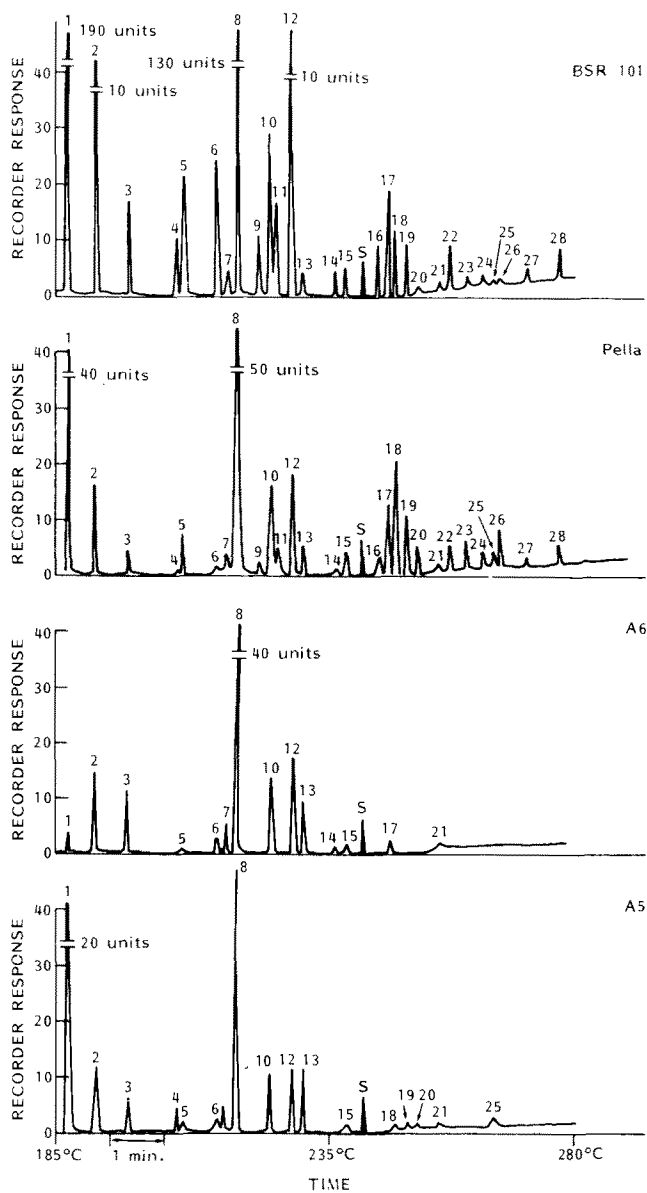


FIG. 1. Chromatograms of TCPH-derivatives from soybean oils after 28 C storage. Peak S is the internal standard, 2-nonanone. GC capillary column is 30-m SPB-1, attenuator 16, electrometer 10⁻¹².

subtraction of the blank are presented in Figure 2. The identities and quantities of the TCPH are shown in Table 5. As with storage at 28 C, many of the compounds found in the commercial varieties, Hardin and BSR 101, were missing in A5 and A6. Hexanal was the most abundant compound found in all the oils, with the amounts occurring in oils in the order: A6 < A5 < Hardin < BSR 101. The order of abundance of the rest of the carbonyls differed among the oils. Fresh A6 contained about the same amount of 18:3 as Pella and Hardin, but A6 had a lower carbonyl content than the other two oils.

The amounts of most of the carbonyls formed at 60 C were lower than the amounts formed at 28 C. White and Hammond (8) also found smaller amounts of carbonyls produced during storage of SBO at 60 C as compared with storage at 28 C. Other researchers (7)

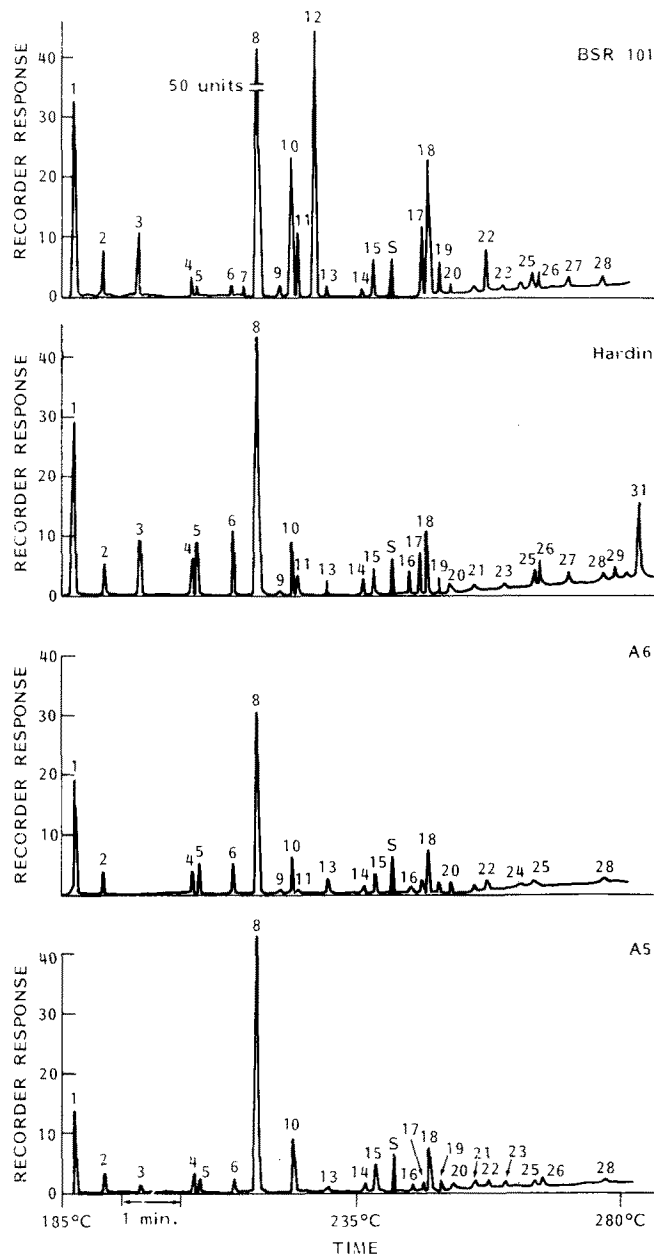


FIG. 2. Chromatograms of TCPH-derivatives from soybean oils after 60 C storage. Peak S is the internal standard, 2-nonanone. GC capillary column is 30-m SPB-1, attenuator 16, electrometer 10⁻¹².

have noted that chemical differences in oxidative stability were not as distinct after 60 C storage as compared with 28 C storage.

The amounts of many of the carbonyls found in the oils oxidized at 60 C were converted to relative flavor potency by using data of Dixon and Hammond (5) (Table 6). For BSR 101, the four compounds contributing most to flavor intensity were the same at both 28 C and 60 C. Hexanal again contributed the most to intensity of flavor, but 2- and/or 3-hexenal had a similar calculated value. The flavor intensities of 2-heptenal and 2-octenal also were almost equal to each other. The carbonyls having the greatest calculated flavor intensity were the same in Hardin, A5 and A6. At 60 C storage, 2/3-hexenal, a major oxidation product of 18:3, was

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TABLE 3

Amounts^a and Identifications^b of Selected Carbonyls Produced From Soybean Oils After Storage at 28 C for 67 Days

Peak No.	Identification	Soybean oil type			
		PV 28.7 A5	PV 28.3 A6	PV 64.3 Pella	PV 100.6 BSR-101
1	acrolein	61.6	4.1	80.3	237.0
2	propanal	12.3	14.9	16.5	52.2
3	butanal	6.6	11.4	4.7	18.0
4	pentanal	4.7	-	0.8	10.7
5	unknown	2.1	0.1	7.5	21.4
6	2,4- <i>t,t</i> -pentadienal	1.7	3.3	1.7	24.7
7	2- <i>t</i> -pentenal	0.8	5.7	2.4	1.6
8	hexanal	47.3	81.9	94.7	178.0
9	2-heptanone	-	-	2.0	11.0
10	unknown	10.4	13.9	16.4	29.0
11	unknown	-	-	2.1	10.9
12 ^c	2- <i>t</i> /3- <i>c</i> -hexenal	11.4	17.7	18.0	58.1
13	2,4- <i>t,t</i> -hexadienal	11.7	9.6	5.4	4.2
14	octanal	-	0.7	1.1	4.8
15	2- <i>t</i> -heptenal	1.1	1.7	4.4	5.5
16	2,4- <i>t,t</i> -heptadienal	-	-	3.2	9.7
17	nonanal	-	2.5	12.9	19.0
18	2- <i>t</i> -octenal	1.6	-	20.9	12.3
19	2,4- <i>t,t</i> -octadienal	1.0	-	10.9	9.3
20	decanal	0.2	-	5.1	0.4
21	2- <i>t</i> -nonenal	0.4	1.5	1.2	1.9
22	2,4- <i>t,t</i> -nonadienal	-	-	4.5	8.2
23	undecanal	-	-	5.2	1.7
24	2- <i>t</i> -decanal	-	-	2.5	2.0
25	2,4- <i>t,t</i> -decadienal	1.2	-	3.6	0.8
26	dodecanal	-	-	6.7	1.1
27	2- <i>t</i> -undecenal	-	-	1.6	2.2
28	2,4- <i>t,t</i> -dodecadienal	-	-	3.1	5.3

^aAmounts listed as ppm of TCPH-derivative in soybean oil.

^bCompounds were tentatively identified by relative retention times.

^cIt has not been established whether 3-*c*-hexenal would rearrange to 2-*t*-hexenal in this procedure. If not, 3-*c*-hexenal TCPH probably would migrate with the TCPH of hexenal.

TABLE 4

Carbonyls Important to Flavor^a in Oils Stored at 28 C for 67 Days

Rank	Soybean oil type			
	A5	A6	Pella	BSR-101
1	hexanal ^c	hexanal ^c	hexanal ^c	hexanal ^c
2	2/3-hexenal ^d	2/3-hexenal ^d	2/3-hexenal ^d	2/3-hexenal ^d
3	2,4-hexadienal	2,4-hexadienal	2-heptenal ^{b,c}	2-heptanone ^c
4	2-heptenal ^{b,c}	2-heptenal ^{b,c}	2-octenal ^{b,c}	2-heptenal ^{b,c}
				2-octenal ^{b,c}

^aAmounts of carbonyls converted into flavor intensity by using data of Dixon and Hammond (5).

^bOxidation product of 18:1.

^cOxidation product of 18:2.

^dOxidation product of 18:3.

TABLE 5

Amounts^a and Identifications^b of Selected Carbonyls Produced From Soybean Oils After Storage at 60 C for 8 Days

Peak No.	Identification	Soybean oil type			
		PV 38.7 A5	PV 40.2 A6	PV 45.0 Hardin	PV 50.0 BSR-101
1	acrolein	14.0	19.4	29.3	33.0
2	propanal	3.7	4.0	5.5	7.9
3	butanal	1.4	-	9.8	10.9
4	pentanal	3.5	4.5	6.3	3.6
5	unknown	2.7	5.3	9.3	2.0
6	2,4- <i>t,t</i> -pentadienal	2.5	5.1	10.9	2.0
7	2- <i>t</i> -pentenal	-	-	-	2.0
8	hexanal	43.3	30.9	43.5	91.9
9	2-heptanone	-	0.7	0.6	2.2
10	unknown	9.3	6.1	9.0	23.4
11	unknown	-	1.0	2.4	10.4
12 ^c	2- <i>t</i> /3- <i>c</i> -hexenal	-	-	-	44.6
13	2,4- <i>t,t</i> -hexadienal	0.5	2.3	2.3	2.0
14	octanal	0.9	1.4	2.9	1.5
15	2- <i>t</i> -heptenal	4.9	3.3	4.9	6.4
16	2,4- <i>t,t</i> -heptadienal	1.1	1.3	4.0	-
17	nonanal	1.9	2.3	7.6	11.7
18	2- <i>t</i> -octenal	7.9	5.9	10.5	22.1
19	2,4- <i>t,t</i> -octadienal	1.5	1.7	2.2	5.6
20	decanal	0.8	1.2	1.3	1.2
21	2- <i>t</i> -nonenal	0.9	0.5	0.6	0.3
22	2,4- <i>t,t</i> -nonadienal	2.7	1.2	-	6.8
23	undecanal	0.4	-	0.7	0.9
24	2- <i>t</i> -decenal	-	0.5	-	0.7
25	2,4- <i>t,t</i> -decadienal	0.4	0.6	2.0	1.3
26	dodecanal	0.5	-	4.1	1.3
27	2- <i>t</i> -undecenal	-	-	2.1	1.3
28	2,4- <i>t,t</i> -dodecadienal	0.4	-	1.1	1.3
29	2- <i>t</i> -dodecenal	-	-	1.5	-
30	unknown	-	-	0.9	-
31	unknown	-	-	16.3	-

^aAmounts listed as ppm of TCPH-derivative in soybean oil.

^bCompounds were tentatively identified by relative retention times.

^cIt has not been established whether 3-*c*-hexenal would rearrange to 2-*t*-hexenal in this procedure. If not, 3-*c*-hexenal TCPH probably would migrate with the TCPH of hexenal.

TABLE 6

Carbonyls Important to Flavor^a in Oils Stored at 60 C for 8 Days

Rank	Soybean oil type			
	A5	A6	Hardin	BSR-101
1	hexanal ^c	hexanal ^c	hexanal ^c	hexanal ^c
2	2-heptenal ^{b,c}	2-heptenal ^{b,c}	2-heptenal ^{b,c}	2/3-hexenal ^d
3	2-octenal ^{b,c}	2-octenal ^b	2-octenal ^{b,c}	2-heptenal ^{b,c}
4	octanal ^b	octanal ^b	2-octenal ^b	2-octenal ^{b,c}

^aAmounts of carbonyls converted into flavor intensity by using data of Dixon and Hammond (5).

^bOxidation product of 18:1.

^cOxidation product of 18:2.

^dOxidation product of 18:3.

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important to flavor only in the high-18:3 oil (BSR 101).

After storage at both 28 C and 60 C, there were many compounds present in large quantities; however, these were not always the compounds that were most important to flavor if relative flavor potencies were considered. The oils contained more 2-octenal than 2-heptenal, but according to the relative flavor potencies, 2-heptenal contributed more to flavor than 2-octenal. According to these flavor potencies, the threshold value also did not predict a compound's flavor importance at higher concentrations. The reported threshold values of 2,4-nonadienal and 2-heptenal are 0.005 ppm and 0.042 ppm, respectively (5). But even in oils containing more 2,4-nonadienal than 2-heptenal, the relative flavor potency of 2-heptenal was greater than that of 2,4-nonadienal. This was true in both storage tests. Likewise, nonanal has a lower threshold value than 2-heptenal (0.011 ppm vs 0.042 ppm) (5). But even in oils that contained more nonanal than 2-heptenal, the relative flavor potency of nonanal was less than that of 2-heptenal.

In the current study, hexanal was the most abundant product formed and also the major contributor to flavor (Tables 4,6). Because hexanal formation is attributed to the autoxidation of 18:2 (1,6), the main contribution to flavor would be from 18:2. Other studies and reviews of SBO oxidation (12-14) have shown high correlations between hexanal content and flavor scores of oxidized vegetable oils. Another contributor to flavor, 2/3-hexenal, arises from the oxidation of 18:3 (1,3). Even so, 2/3-hexenal was an important flavor compound formed in the low-18:3 oil (A5) at 28 C storage. This was not true at 60 C storage. Two carbonyls having high relative flavor potencies, 2-heptenal and 2-octenal, are minor products of the autoxidation of both 18:1 and 18:2 (1). Thus, much of the autoxidized flavor in these SBO came from 18:1, 18:3 and, especially, 18:2.

At both 28 C and 60 C storage, A5 and A6 contained fewer carbonyls and, according to PV and CD, were more stable. Sensory evaluation of A5 and A6 stored at 28 C was inconclusive, but, at 60 C storage, A5 and A6 tended to be more bland than BSR 101, though the differences were not generally significant.

SBO generally has poorer flavor stability than oils with similar 18:2 contents. Many reasons have been proposed for this difference. Smouse and Chang (15) suggested that the high 18:3 content of SBO catalyzes the autoxidation of 18:2 and also may alter the decomposition pattern of its hydroperoxides. A study by Raghuvver and Hammond (16) supported this idea. They found that the FA in SBO oxidized at different rates than the pure methyl esters of the same FA. Cosgrove et al. (17) found that the rates of oxidation were affected by incorporation into a triglyceride. They proposed that the aggregation of lipid molecules, the ester functionality and the close proximity of the FA

chains all affected the rate of oxidation. The glyceride structure of the fat or oil also affected the rate of oxidation, according to Lau and Hammond (18). The effect of a mixed triglyceride on the breakdown products is unknown.

After storage at 28 C or 60 C, A5 and A6 contained fewer carbonyls than oils from commercial varieties. The types and amounts of volatiles produced depended upon both storage temperature and the type of SBO. More of the carbonyls that were identified arose from 18:2 rather than 18:3, particularly at 60 C storage, because 2/3-hexenal was not found in the lower-18:3 oils.

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